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New Concepts in Assessing Sickle Cell Disease Severity

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Vasoocclusion leads to pain, chronic organ damage, and a decreased life expectancy in patients with sickle cell disease. Therapeutic options for sickle cell disease have usually been evaluated according to their capacity for reducing the frequency of vasoocclusive crises requiring clinical attention. However, the frequency of vasoocclusive crises is not representative for the rate of accumulating organ damage in most sickle cell patients. This implies that the frequency of vasoocclusive crises needn't correlate with disease severity and, although being of importance, cannot solely serve as a parameter of treatment efficacy. Therefore, additional new objective parameters are needed to effectively study the vasoocclusive process in sickle cell disease. Several studies show that intricate adhesive interactions between (red) blood cells, plasma components, and endothelium play a crucial role in the pathophysiology of sickle cell vasoocclusion, offering new potential parameters to effectively assess disease severity as well as new therapeutical targets in the near future. Whether these adhesive mechanisms involve the causes or the effects of vasoocclusion will be determined if their inhibition, by interventive measures, results in therapeutic benefits. Am. J. Hematol. 58:61–66, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

Sickle cell disease is a term encompassing a distinct class of hemoglobinopathies. A single point mutation in the sixth position of the β-globin gene, resulting in the substitution of valine for glutamic acid, leads to the replacement of normal hemoglobin (HbA) by sickle-hemoglobin (HbS). The term sickle cell disease usually refers to the homozygous state (HbSS), but may also include double heterozygous states (in which other hemoglobin disorders are inherited together with HbS), such as HbSC (sickle-C disease), combinations of HbS and β-thalassemias, and other less common variants. Sickle cell disease is a heterogenous disorder, with clinical manifestations including chronic hemolysis, increased susceptibility to infections, and recurrent painful vasoocclusive crises that ultimately lead to chronic ischemic organ damage [1,2].

This review seeks to emphasize the limitations of painful crisis frequency as a parameter of disease severity. Based upon the current insights into the pathophysiology of sickle cell vasoocclusion, we discuss immunological, hematological, and biochemical factors that may serve as potential parameters to effectively assess disease severity and to evaluate treatment efficacy. These factors may also provide potential targets for future therapy.

PAINFUL CRISES AND DISEASE SEVERITY

The process of microvascular occlusion underlies much of the morbidity and mortality associated with sickle cell disease [1,2]. Recurrent self-limiting episodes of vasoocclusion, known as painful crises, are the hallmark of sickle cell disease. A painful crisis is defined as an event characterised by musculoskeletal (usually juxta

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articular) and/or visceral pain not otherwise explained and mostly associated with mild pyrexia and the passage of dark or red urine [3]. These painful crises are the most frequent cause of sickle cell disease-related hospitalization and crisis frequencies higher than 3 per year have been shown to be a risk factor for early death [4,5]. The frequency of painful crises is generally regarded as the most important parameter of sickle cell disease severity, and therefore is widely used as the main indicator of therapy efficacy.

Crisis frequency is usually determined by scoring only those episodes leading to a hospital or emergency room visit [6]. Interestingly, most sickle cell patients do not frequently suffer from painful crises requiring clinical attention. Platt et al. followed 2412 HbSS patients for 9 years and showed that patients with 3 or more crises per year represent only 5.2% of the studied population, accounting for 32.9% of all clinically observed crises. Of the population of HbSS patients analyzed, 39% had no clinical episodes and about 40% had up to 1 clinical episode per year. Similar results were obtained in HbSC disease and other sickle syndromes [5]. In another study, 84% of hospitalized crises episodes were accounted for by 23% of HbSS patients [4]. The authors also showed that 40% of the patients had crises in clusters, interspersed between long periods in which no clinical attention was sought for treatment of painful crises. Observation of 391 sickle cell anemia patients during 6.4 years showed that approximately 36% of the studied population did not have a painful crisis that required clinical attention [7]. These reports exemplify the fact that sickle cell patients with frequent painful crises leading to a hospital or emergency room visit, comprise only a relatively small subgroup of the total population.

Even though most sickle cell patients do not frequently suffer from painful crises leading to clinical care, the contribution of pain in sickle cell disease-related morbidity is considered to be of great significance in the majority of patients. The incidence of pain-induced morbidity in sickle cell disease is significantly underestimated when solely based upon clinically gathered data [6]. As noted by Pearson et al. and Platt et al. most episodes of pain respond to simple analgesia and oral rehydration and, therefore, do not require special clinical attention, hence remaining ''invisible'' to the clinician [8,9]. The importance of this observation is reflected by the fact that life expectancy for patients with less than 3 painful crises leading to clinical attention per year is still significantly below the life expectancy of the general black population. In this group, which comprises the majority of HbSS patients, no further subdivision of clinical painful crises in relation to life expectancy can be observed. Between the ages of 38 and 54 years, survival probability seems to be even lower in patients seeking clinical care for less than 1 painful crisis per year as compared to patients who receive clinical attention for 1 to 3 painful crises per year [5,10].

There is no doubt that microvascular occlusion accounts for the greatest part of the clinical spectrum of sickle cell disease. It seems probable that, even in clinically non-symptomatic patients, active ''silent'' vasoocclusion occurs, resulting in characteristic sickle cell disease-related complications. For example, in relatively well-oxygenated organs such as the lungs, scarring of smaller airways (caused by long-term microvascular occlusion) is a common finding without the occurrence of previous acute chest syndromes [3]. Therefore, it does not seem unreasonable to presume that the process of silent vasoocclusion may diminish the quality of life of sickle cell patients (such as is the case in the mentioned example of the lungs by inducing chronic hypoxemia). Accordingly, the following classification is proposed to effectively describe vasoocclusion in sickle cell disease:

- painful crises requiring hospital or emergency room care (*clinical vasoocclusion*);
- painful episodes that do not require clinical care and are treated at home (*non-clinical vasoocclusion*); and
- low-grade chronic vasoocclusion without classical vasoocclusive symptoms, but nevertheless accumulating to irreversible organ damage in the long run (*silent vasoocclusion*).

PAIN, VASOOCCLUSION, AND ORGAN DAMAGE

Pain in sickle cell disease is caused by vasoocclusion, leading to damage of vasculature and organs [1,2]. However, there is no correlation between the frequency of clinical vasoocclusion and vasculopathy of the spleen, the central nervous system, the kidney, or the retina [4]. Furthermore, the frequency of clinical vasoocclusion as an objective parameter is questionable as the number of previously experienced episodes is shown to be a powerful predictor of a next episode, overshadowing influences such as therapeutic interventions [11]. It can be reasonably argued that the mere frequency of clinical vasoocclusion, while still being one of the most important parameters of disease activity, should not be taken as the only indicator of the severity of the pathological process. Therefore, one may have to be very cautious to label patients with few episodes of clinical vasoocclusion as mild cases of sickle cell disease. Even the apparently mildly affected patient will, at some point in life, begin to develop clinical manifestations of disease mainly due to the accumulation of chronic vascular damage throughout the years (partly as a result of silent vasoocclusion) [4].

The shortcomings of using the frequency of clinical vasoocclusion as the most important parameter for disease severity is further exemplified in evaluation of therapeutic interventions. Results of several studies re-

garding treatment of sickle cell patients with hydroxyurea, which over the last years has been regarded as one of the most promising therapies for sickle cell disease, showed a reduction of clinical vasoocclusion in some, but not all, enrolled patients [12–15]. In these studies, only patients with at least 3 episodes of clinical vasoocclusion in the last year were enrolled, leading to nonrepresentative results for the general sickle cell population. More importantly, basing therapy efficacy upon a reduction of clinical vasoocclusion clearly is not accurate as the frequency of clinical vasoocclusion does not represent the rate of developing complications from accumulating organ damage in the majority of patients.

Because of the advent of efficacious, readily accessible, and low-tech therapeutic modalities for the acute sickle cell syndromes (blood transfusions, antibiotics, analgesics), the mean life expectancy for this group of patients is on the rise. As sickle cell patients are growing older, the diminishing effect of chronic organ damage on the quality of life should become a primary concern, a view underlined by sickle cell patients in a recent report from the St. Thomas Hospital in London [4,10,16–19]. Therefore, the concept of silent vasoocclusion is becoming an increasingly important phenomenon. For accurate monitoring of disease severity and therapy efficacy, future management (as well as studying) of sickle cell disease should take this concept into account. However, how are we to determine whether silent vasoocclusion is occurring, let alone to what extent? Evaluation of current insights into the pathophysiology of sickle cell vasoocclusion offers several potential objective parameters, which could become useful instruments to assess silent vasoocclusion, as well as new targets for therapeutic intervention.

CURRENT CONCEPTS IN THE PATHOPHYSIOLOGY OF SICKLE CELL VASOOCCLUSION

Suitable (clinical) outcome measures of disease, which accurately reflect disease activity, are imperative for identification and development of beneficial therapeutic strategies. For sickle cell disease, new outcome measures are needed to complement clinical vasoocclusion for accurate monitoring of disease severity and therapy efficacy. Even though the roles of adhesion molecules, endothelial cell abnormalities, cytokines, and the various types of blood cells in the pathophysiology of sickle cell vasoocclusion have not been fully elucidated, several potential new tools for effective disease monitoring could be emerging. While conceding the fact that in studies regarding the kinetic profiles of these factors, it is often impossible to distinguish between cause and effect, it still seems worthwhile to summarize the state of the art in this field of research. Due to the complexity of multiple interactions between the aforementioned factors, cause-effect relationships will inevitably often be mutual and bidirectional. The identification of the factors at play, may they be either causes or effects, is of primordial concern and in the best interest of this group of patients. The ultimate proof that some process is of causal importance in the vasoocclusive event, would be that its inhibition by some therapeutic intervention either aborts an ongoing sickle cell crisis or prevents the occurrence of long-term irreversible organ damage.

The important role of adhesive events in sickle cell vasoocclusion was already evident in 1980 when Hebbel et al. showed that adherence of sickle red blood cells strongly correlates with the clinical manifestations of the disease due to vasoocclusion [20]. Elegant experiments have shown the importance of young red blood cells in these events [21]. Several reports have shown that next to erythrocytes, endothelial cells and leucocytes play essential roles in the onset and maintenance of vasoocclusion in sickle cell disease [22–29]. Cytokines, coagulability factors, and atherogenic factors (such as homocysteine) also seem of importance during these events [30–37].

Soluble adhesion molecules are considered to be released especially by endothelial cells upon cytokine stimulation [38–40]. The observation that clinically asymptomatic HbSS and HbSC patients have enhanced levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) is therefore indicative of a constant activation of endothelial cells [29,38–41]. When activated, endothelial cells increase their surface expression of adhesion molecules, and thereby promote sickle red blood cell and leucocyte adherence [29,32,39,42,43]. This impedes the microcirculation and increases the transit-time of sickle red blood cells, probably resulting in (or contributing to) silent vasoocclusion with ischemic organ damage in the long run. During a vasoocclusive crisis a further increment of sVCAM-1 is observed, suggesting that a painful crisis (clinical vasoocclusion) should be considered as an exacerbation of a chronic process (silent vasoocclusion) [41]. A recent study shows the importance of these observations, as the reduction of clinical vasoocclusion in patients on hydroxyurea therapy is, at least in part, based upon a reduction of adhesive events [44].

Even though the true role of soluble adhesion molecules still remains unclear, their increased levels may provide useful diagnostic or prognostic information in various diseases. Levels of sVCAM-1 have been shown to correlate with disease activity in several inflammatory disorders [38,43,45]. For example, sVCAM-1 levels are reported to be elevated in patients with Wegener's granulomatosis [45]. In patients with widespread disease, sVCAM-1 levels were highest (as compared to patients with limited organ involvement), and sVCAM-1 levels increased significantly prior to a clinical relapse. Because

sickle red blood cell adhesive events especially involve the binding of endothelial VCAM-1 to its counter receptor, namely the very late activating antigen-4 (VLA-4), the extent of VCAM-1 expression on the endothelium is a possible indicator of the extent of vasoocclusion [22–25,29]. Large prospective follow-up studies will have to prove whether sVCAM-1 can indeed serve as an indirect parameter for disease severity.

Several groups have postulated neutrophilic granulocytes to play an essential role in sickle cell vasoocclusion [27,28]. Adherence of neutrophils to endothelium could be an important factor in the initiation and propagation of vasoocclusion in sickle cell disease [46]. Furthermore, adhesion of neutrophils to vascular endothelium has been shown to result in endothelial damage in various inflammatory diseases [29]. The contribution of neutrophils at the onset of clinical vasoocclusion is further supported by the elevated levels of the potent neutrophil activator and chemoattractant interleukin-8 (IL-8) (Duits, unpublished data), and by the fact that a significant decrease in neutrophil counts strongly correlates with a decrease in the frequency of clinical vasoocclusion [47,48]. As blood leucocyte counts correlate with clinical severity in sickle cell disease, further elucidation of the role of neutrophils (and their activating proteins) in sickle cell vasoocclusion may also give rise to potential parameters for monitoring disease activity [10].

Endothelial activation (and ensuing damage) as well as hypercoagulability could also result from biochemical factors such as elevated plasma homocysteine levels, which are encountered in patients with sickle cell disease [36,37,49]. A recent study shows a possible positive correlation between the occurrence of stroke and plasma homocysteine levels in sickle cell disease [37]. Further investigations should prove whether elevated plasma homocysteine levels are useful to identify patients at high risk for complications.

POTENTIAL IMPACT ON SICKLE CELL DISEASE TREATMENT

Currently, a great deal of attention is being focused on the exciting new avenues in sickle cell disease described above. Since these factors seem of fundamental importance in all degrees of vasoocclusion, treatment options based upon these parameters should be applicable to all sickle cell patients, independently of the frequency of clinical vasoocclusion. Therapies primarily aimed at reducing silent vasoocclusion might prove to be very effective in reducing sickle cell disease-related morbidity. As vasoocclusive complications are observed as early as the first year of life in sickle cell disease, reduction or prevention of accumulating vascular and organ damage by targeting silent vasoocclusion is to be started at an early age and to be continued throughout life, ideally by therapies lacking adverse (long-term) side effects [50]. Elucidating the exact nature of the cytokines and adhesion molecules involved is, therefore, of great importance.

The nuclear transcription factor NF-κB regulates the expression of several cytokines (such as IL-8) and adhesion molecules (such as VCAM-1) [51]. The enhanced sVCAM-1 and IL-8 levels observed in sickle cell patients indicate an NF-κB regulated inflammatory state. As significant information has been obtained on the regulation of gene expression, disease intervention could be aimed at protein or even gene activity. The increasing evidence that glucocorticoids inhibit the actions of NF-κB might explain the decreased duration of an episode of clinical vasoocclusion when methylprednisolone is administered [51,52]. Recently Wolters et al. conducted a pilot study that showed low dosage coumarine therapy to safely reduce the pre-thrombotic state in sickle cell patients [53]. As NF-κB activity can simply be down-regulated by coumarine derivatives, large prospective studies regarding the influence of coumarine derivatives on hypercoagulation and NF-κB activity in sickle cell patients are warranted [54]. Currently, a randomized, double-blind, placebo-controlled pilot study is being conducted to test the efficacy of low-dose coumarine derivatives in sickle cell patients (Duits, personal communication). A quality of life questionnaire is being used to ensure a valid interpretation of therapy efficacy for all categories of sickle cell patients [55]. In therapeutic trials with representative patient populations, episodes of manifest clinical vasoocclusion will be relatively scarce. The use of a simple aid like a validated quality of life assessment scale would objectively mirror the patient's well-being, giving the clinician a valuable tool to detect (more subtle) therapeutic effects on all types of vasoocclusion.

Based upon the absence of manifest hematological signs of folate deficiency, sickle cell patients are considered to have adequate folate levels [56,57]. In Jamaica, folate supplementation did not appear to reduce the occurrence of clinical vasoocclusion and is discouraged [18,57]. However, our group encountered elevated homocysteine levels in clinically folate sufficient sickle cell patients, indicating a sub-optimal folate status (Van der Dijs, unpublished data) [58]. Folate supplementation safely reduces elevated homocysteine levels and thereby appears to ameliorate endothelial dysfunction [59]. Even though folate supplementation seems to have no effect on the occurrence of clinical vasoocclusion, the accumulating detrimental effects of homocysteine on the endothelium could easily be avoided. The impact of folate supplementation on silent vasoocclusion should therefore be analyzed [60].

CONCLUSION

Whereas the majority of sickle cell patients do not suffer from frequent episodes of clinical vasoocclusion, non-clinical and silent vasoocclusion leading to chronic organ damage are of great significance in most sickle cell patients. Therefore, the frequency of clinical vasoocclusion should not be the sole parameter for sickle cell disease severity, nor can it serve as an accurate parameter of therapy efficacy in all patients. Current concepts show prominent, although not yet fully unraveled, roles for red and white blood cells, endothelium, cytokines, and adhesion molecules in all degrees of sickle cell vasoocclusion. Critical analysis should provide not only potential objective parameters to assess disease severity and to monitor therapy efficacy, but future targets for therapeutic intervention as well.

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